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COLLAGEN SOLUBILITY AND CALCIUM CONCENTRATION AND THEIR EFFECTS ON TENDERNESS IN THE *M. LONGISSIMUS LUMBORUM*

A Thesis

by

DANIEL PHILLIP GENHO

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2009

Major Subject: Animal Science

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Approved by:

Chair of Committee,	Jeffrey W. Savell
Committee Members,	Rhonda K. Miller
	Stephen B. Smith
Head of Department,	Gary R. Acuff

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ABSTRACT

Collagen Solubility and Calcium Concentration and Their Effects on Tenderness in the *M. longissimus lumborum.* (December 2009)

Daniel Phillip Genho, B.S., Utah State University

Chair of Advisory Committee: Dr. Jeffrey W. Savell

Strip steaks from the McGregor genome project were used to evaluate the effects of sarcomere length, myofibrillar fragmentation index, 3 h postmortem pH, 24 h postmortem pH, marbling, electrical stimulation (ES), sarcoplasmic free calcium concentration, and collagen characteristics on tenderness as measured by Warner-Bratzler shear force (WBS). The WBS values were measured prior to this project so the animals were able to be separated into "tender" and "tough" groups using a WBS value of 30 N as the separating point, steaks with a WBS value less than 30 N being "tender" and the others being "tough".

It was found that ES sides had lower WBS values, however, "tough" steaks showed a greater response to ES than "tender" steaks. ES sides also had higher sarcoplasmic free calcium concentration and lower 3 h postmortem pH. Tenderness is best predicted by treatment (ES versus NON-ES), however, there is some efficacy in using total collagen and collagen solubility in conjunction with treatment.

DEDICATION

This thesis is dedicated to my family. Particularly to my parents, Dr. Paul and Meredith Genho, who instilled in me a love for learning at an early age.

ACKNOWLEDGEMENTS

I would like to thank the members of my committee, Dr. Jeff Savell, Dr. Rhonda Miller, and Dr. Steve Smith for their help in developing and executing this project, as well as, the knowledge they imparted to me not only in the classroom but also in the time I've spent working with them individually.

Thanks to the student workers, graduate students and the workers at the RMSTC for the work they've done in the past to obtain some of the data used in this project. Thank you to Sharron Nowak for her guidance in the laboratory and to Ashley Haneklaus for her help in obtaining supplies and working with the technicalities of research.

A special thanks to Dr. Rhonda Miller for working closely with me in the statistical analysis and measuring collagen solubility. Thanks to Eric Matteaur and Dr. Kristin Nicholson for the work they did which allowed me to utilize their data in this project. Another special thanks to Dr. David Forbes and his laboratory, at the Texas Agricultural Experiment Station in Uvalde, Texas, for allowing me to use their equipment to complete the data collection.

Finally I'd like to extend my thanks to my family, particularly my father Dr. Paul Genho for his support and motivation in helping me move forward in my educational pursuits, and my mother Meredith Genho for her never ending love and support. Thanks to my siblings as well, particularly Michael and John who motivated me to work hard and supported me through my endeavors.

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CHAPTER I

INTRODUCTION

Tenderness is a major concern to American meat producers, particularly beef producers; it has become an issue because of the problems with variation in tenderness in the United States beef supply. For this reason, it is imperative to understand tenderness and the biochemical systems behind it. This concern has led to the development of several "Tender" labeled beef programs (Hilton, Gentry, Allen & Miller, 2004).

The overall satisfaction of a consumers eating experience is a sum of the juiciness, flavor and tenderness of the beef (Lorenzen et al., 1999; Neely et al., 1998) Tenderness is of primary concern because of the limited variability in juiciness and flavor in U.S. beef (Lorenzen et al., 1999; Neely et al., 1998), with tenderness being the single most important factor in affecting the perceived taste of beef. Many approaches have been taken in an effort to better understand the factors contributing to tenderness. Some of these factors include but are not limited to, marbling (Smith et al., 1985), myofibrilar fragmentation index (Olson & Parrish, 1977), calpain/calpastatin levels (Koohmaraie, 1988), sarcomere length (Koohmaraie, Seideman, Schollmeyer, Dutson & Babiker, 1988b), electrical stimulation, muscle pH, calcium concentration (Ji & Takahashi, 2006) and collagen content and solubility of the muscle (Cross, Carpenter & Smith, 1973).

This thesis follows the style of *Meat Science.*

The issue in the past has been the inability to account for variability in tenderness. Many studies have been designed and run to correlate marbling with tenderness, but this has yielded varied results. Blumer (1963) was able to explain about 5% of the variability in tenderness using marbling, while a later study was able to account for about 24 to 34 % of the variation in tenderness, flavor and overall palatability in A maturity loins steaks with marbling (Smith et al., 1985). Marbling alone is unable to account for a high percentage of the variation in tenderness.

Myofibrillar fragmentation index (MFI) is a measure used to indicate the incidence of postmortem proteolysis (Olson & Parrish, 1977; Parrish, Young, Miner & Andersen, 1973). MFI has been show to relate strongly to tenderness (Olson & Parrish, 1977; Parrish et al., 1973). There is a correlation between MFI and both sensory panel tenderness ratings and Warner-Bratzler shear force values (Olson & Parrish, 1977). Olson and Parrish (1977) accounted for about 50% of the variation in tenderness with myofibrillar fragmentation index.

Other measurements have been used to try to account for the variation in tenderness of beef. Calpain/calpastatin levels and activity has received much attention, but conflicting studies have yielded varied results. Several studies have indicated that there exists a relationship between calpain activity and tenderness (Koohmaraie, Babiker, Merkel & Dutson, 1988a; Koohmaraie, Babiker, Merkel & Dutson, 1992; Morgan, Wheeler, Koohmaraie, Savell & Crouse, 1993), whereas others have disagreed with the results from these researchers (Boehm, Kendall, Thompson & Goll, 1998; Ji &

Takahashi, 2006), making calpain/calpastatin questionable for accounting for the variation in tenderness between animals.

Sarcomere length also has been associated with tenderness (Herring, Cassens & Briskey, 1965). Herring et al. (1965) correlated sarcomere length to fiber diameter and to tenderness, yet they were only able to use these measurements to explain about 12% of the variation of tenderness.

Electrical stimulation of beef carcasses has been used to decrease the occurrence of cold shortening and improve tenderness, by 34-45% (Ferguson, Jiang, Hearnshaw, Rymill & Thompson, 2000). This change, although significant, does not have any relationship with inherent tenderness differences that exist, because electrical stimulation is a postmortem intervention to improve tenderness, much like blade tenderization.

Muscle pH has been associated with tenderness values as expressed by Warner-Bratzler shear force values (Yu & Lee, 1986). These relations have been demonstrated in the strongest fashion in excessively high pH, > 6.3, but there has been some relation in pH below 5.8 as well (Yu & Lee, 1986).

Efforts to explain variation in tenderness have been numerous. Unfortunately most of the work still fails to completely account for high amounts of variation in tenderness, with much of the work in similar areas producing either varying or confounding results.

Data in the past have shown that sarcoplasmic calcium concentration and collagen solubility both play a role in tenderness; however, these data have not been

correlated, or tested on the samples from the same animals, to account for the actual effect each plays on tenderness.

The purpose of this project was to use sarcoplasmic free calcium concentration, and collagen characteristics to explain variation in tenderness. Also to use inn combination with these, sarcomere length, MFI, electrical stimulation and carcass data to predict tenderness, defined as Warner-Bratzler shear force, in electrically stimulated and non-electrically stimulated steaks from the same carcass. Tenderness variation was induced by selecting carcasses that vary in tenderness from specific families from the McGregor Genome study as reported by Metteaur (2008) and Nicholson (2008).

CHAPTER II

LITERATURE REVIEW

Collagen is a connective tissue protein found in muscle tissues, and is made up of tropocollagen molecules that overlap one another. Connective tissue is found in many forms in the body, and is a component of tendons and ligaments, fat, blood, vesicles and muscles as epimysium, perimysium and endomysium. Perimysium accounts for 90% of the total connective tissue in muscles (McCormick, 1999) and is the connective tissue of major concern in reference to tenderness.

Collagen has an unusually high amount of the amino acid glycine, and comprise out of every three amino acids in collagen (Aberle, Forrest, Hedrick & Merkel, 2001). Collagen also contains a unique imino acid called hydroxyproline, which is used to measure total collagen concentration and collagen solubility in a muscle (Woessner, 1961).

Varying collagen contents have been reported for muscles, for example 1.5-10% of the dry weight (Lepetit, 2008) and a connective tissue concentration of 1-4% of the dry weight (Taylor, 2003). Normal collagen concentration in *m. longissimus lumborum* has been found to be between 3.24 and 3.38 mg/g (Culler, Parrish, Smith & Cross, 1978).

Variation between tenderness values of muscles may be related to the collagen content (Mitchell, Hamilton & Haines, 1928; Ramsbottom, Standine & Koonz, 1945). Muscles of locomotion have higher connective tissue concentrations and are generally

tougher while muscles of support have lower connect tissue amounts and are more tender (Ramsbottom et al., 1945).

Animal sex has been examined as a factor in collagen amount. Meat from heifer and steer beef was found not to differ in collagen amount (Mitchell et al., 1928). Bull meat has been shown to have higher connective amounts than steer meat (Crouse, Seideman & Cross, 1983). In sheep, wethers and rams had similar total collagen, yet the percent of soluble collagen was greater for rams than weathers (Maiorano, McCormick, Field & Snowder, 1993).

Collagen solubility

Collagen can be subdivided into heat labile and heat stable portions (Horgan, Jones, King, Kurth & Kuypers, 1991). The heat labile collagen melts, or gelatinizes, in the presence of heat. The temperature of gelatinization has been reported at temperatures from 60-63ºC (Leander, Hedrick, Brown & White, 1980) to 70ºC (Pohlman, Dikerman, Zayas & Unruh, 1997).

Taylor (2003) cites Culioi (1995) as showing that collagen solubility between animals of similar age, sex and nutrition can still vary more than two fold. Percent of the collagen that is soluble has been shown to correlate with tenderness (Cross et al., 1973; Herring, Cassens & Briskey, 1967). However, the amount of soluble collagen only played a significant effect in predicting of amount of connective tissue ratings by a sensory panel (Cross et al., 1973).

Diet also plays a role on the amount of connective tissue in an animal. Miller, Cross, Crouse and Tatum (1987), showed that the energy content of a diet fed played an

role in the amount of perceivable connective tissue by a sensory panel. High-energy diets had a less perceivable amount of connective tissue than low energy diets ($P < 0.05$). It was also shown that days on feed improved the amount of connective tissue ratings particularly from 0 days to 56 d with continued improvements after that (Miller et al., 1987). Interestingly, there is not a significant decrease in the amount of collagen by feeding, but there is a significant increase in the percent of soluble collagen from 0 to 56 days on feed (Boleman, Miller, Buyck, Cross & Savell, 1996). Aberle, Reeves, Judge, Hunsley and Perry (1981) showed that animals feed a high-energy diet for at least 70 d, after a 153 d low energy diet, show improved collagen solubility over those fed solely a low energy diet for 230 d. Which seems to be in line with data presented by Wu, Kastner, Hunt, Kropf and Allen (1981), who showed that steaks from cattle fed a corn-based diet for 120 d prior to slaughter showed improved collagen solubility over steaks from animals fed solely grass for 120 d prior to slaughter.

Age of the animal does not play a part in the amount of total collagen present in the animal, although there are differences between animals with big age differences (Herring et al., 1967). Yet collagen solubility does decrease with age, and it is the solubility that is most highly related to tenderness (Cross et al., 1973). As an animal ages, collagen becomes less soluble, and inherently tougher, but the amount of total collagen does not increase in the animal (Cross et al., 1973; Herring et al., 1967). Goll, Hoekstra and Bray (1964) found that in veal at 40 days of age cooked at 70ºC, 42% of the collagen was soluble, while only 2% was soluble in beef from animals of 10 years of age.

Collagen cross-linking

The thermal stability of collagen is often associated with the cross-linking of the collagen (Taylor, 2003). Cross-links are connections formed either within or between tropocollagens. These cross-links are generally formed between the amino acids lysine and glysine.

There are many categories of cross-links which are types I-XIV (Reiser, McCormick & Rucker, 1992). These types of cross-links are generally either divalent (two molecules bonded initial cross-links) or trivalent (three molecules bonded mature cross-links) (Eyre & Wu, 2005), with the trivalent being theoretically 1.5 times more functional than the divalent (Lepetit, 2008).

Some cross-linking types are considered thermo-labile and others thermo-stable (Horgan et al., 1991), which correlate generally to the heat soluble and heat insoluble collagens. The most abundant types of tropocollagen present in muscle are type I and III (Lepetit, 2007; Light & Champion, 1984), with type I collagen being less heat stable than type III (Burson & Hunt, 1986a). Cross-linking, much like percent insoluble collagen, increases with the age of the animal (Taylor, 2003).

Collagen effects on tenderness

Collagen does affect tenderness. Lepetit (2008) cites Kopp and Bonnet (1982) as showing that the shear force value of raw beef is correlated with collagen content at a r^2 of 0.81; however, the concern in tenderness is not so much with raw beef as it is with cooked beef.

Prior to heating collagen is what Lepetit (2007) refers to as a "quasi-crystalline structure," this structure is highly elastic. Upon heating to temperatures of 58-65ºC, this structure denatures and contracts and becomes more rubber-like (Lepetit, 2007). Taylor (2003) concluded from his review of multiple studies that from 40-50ºC meat toughens due to water loss and protein gelation; from 50-60ºC tenderization occurs due to the melting of connective tissue; and above 60ºC toughening occurs as muscle fibers harden.

Cross et al. (1973) showed that the percent of soluble collagen in a cooked muscle correlates with the sensory measure of tenderness in that muscle, while the total collagen content did not have the same effect. Varying correlations have been found between tenderness and both total collagen content and collagen solubility. Correlations of total collagen content to tenderness have ranged from 0.66 to 0.82 (Riley et al., 2005) and 0.09 to 0.30 (Serra et al., 2008), because similar feeding strategies and slaughter ages were used in both studies, this marked difference could be due to the different breed types that where used in each study. Riley et al. (2005) used Brahman cattle and Serra et al. (2008) utilized Spanish breeds of cattle. Percent soluble collagen and its correlation with tenderness has ranged from 0.77 to 0.81 (Herring et al., 1967) and 0.01 (Campo et al., 2000). There are also some breed differences between these two studies, Herring et al. (1967) utilized commercial fed cattle from maturity grades A, B and E and was not breed specific. Campo et al. (2000) utilized very specific breeds in order to see the effect of breed on beef quality.

As with the collagen solubility, varying results have been reported as to the effect of cross-linking in collagen on meat tenderness. Light, Champion, Voyle and Bailey

(1985) did not find correlation between type I and type III cross-linking and tenderness. Yet Mairano et al. (1993) found that there was a difference between the type of collagen cross-linking and tenderness values in sheep. There has been some evidence that amount of cross-linking does play a role in tenderness (Bailey & Light, 1989) although this has been highly variable from study to study as well.

Calcium concentration

Calcium also plays an important role in tenderness, as it is involved in the postmortem degradation of muscle proteins. Koohmaraie, Babiker, Merkel and Dutson (1988a) stated that calcium is the mediator of postmortem tenderization. Koohmaraie, Babiker, Merkel and Dutson (1992) demonstrated that calcium is the driving force behind the calpain and calpastatin actions in postmortem beef, which they referred to as CDP or calcium dependent proteases. Varying results and opinions on the actual effect of this CDP reaction on tenderness have been formed, some highly agree with this theory (Koohmaraie et al., 1988a; Koohmaraie et al., 1992; Morgan et al., 1993), while others disregard the theory as improbable because the pH and temperatures at which both m-calpain and μ-calpain are active are markedly different from the actual pH and temeperature of postmortem beef (Ji & Takahashi, 2006; Kanawa, Ji & Takahashi, 2002). Etherington, Taylor and Dransfield (1987) did not find any definite correlation between the rate of aging and calpain levels. So although calcium has been related to the CDP tenderizing activity in the past, the actual role is still unclear and debated.

Calcium in the muscle

Calcium plays an important role in the normal contraction and relaxation of living muscle. According to Seeley, Stephens and Tate (2003), when the action potential has traveled down the axon to the presynaptic terminal, calcium ions are pumped in through a calcium specific pump. This pumping causes the migration of the synaptic vesicles, which contain acetylcholine, to the surface of the synaptic cleft. Upon reaching this cleft, the vesicles open, or rupture, releasing acetylcholine into the synaptic cleft. The acetylcholine activates the sodium pumps on the surface of the sarcolemna and causes an influx of sodium into the muscle cell, which propogates the action potential into the cell. Acetylcholinesterase then digests the acetylcholine into acetic acid and choline. The choline is taken back up by the presynaptic terminal and the calcium in the presynaptic terminal is pumped out. The choline reacts with acetic acid in the terminal to form vesicles filled with acetylcholine so that the entire process can begin again, with the re-influx of calcium upon the arrival of a new action potential.

Aberle and others (2001), explained that the action potential that has crossed the synaptic cleft into the sarcolemna, travels through the sarcolemna to the interior portion of the T-tubules, which causes the release of calcium from the sarcoplasmic reticulum, where it is stored, into the sarcoplasm. This occurs by the action potential depolarizing the dihydropyridine receptor and allowing calcium to flow out. This calcium then is able to bind to the troponin complex, particularly to troponin C, causing a shift in inhibitory troponin I, which shifts tropomyosin and allows for the actin and myosin cross-bridge to

occur and contraction to take place. For contraction to occur, a 10^{-6} to 10^{-5} moles/liter concentration of calcium in the sarcoplasm is necessary.

Aberle and others (2001), also reported that for relaxation the calcium that has been released from the sarcoplasmic reticulum activates a calcium pump in the sarcoplasmic reticulum membrane. Once activated, this pump pulls free calcium out of the sarcoplasm back into the sarcoplasmic reticulum, causing the calcium bound to the troponin complex to release. This only occurs with high amounts of ATP. It takes less calcium for relaxation than it does for contraction, about 10^{-7} moles/liter during relaxation.

Ultimate free calcium concentration in the muscle normally changes from 100nM to 210-230μM during the normal postmortem aging of meat. This is equivalent to changing from 10^{-7} moles/liter to 10^{-4} moles/liter (Ji & Takahashi, 2006). The amount of sarcoplasmic free calcium in the bovine *longissimus* muscle has been found to be as high as 970μM (Parrish, Selvig, Culler & Zeece, 1981).

Calcium effects on tenderness

One theory of calcium mediated tenderization of beef has been developed (Takahashi, 1996; Takahashi, 1999). This theory states that the increase of sarcoplasmic calcium concentration during post mortem aging, by the inability of the sarcoplasmic reticulum to sequester released calcium, to at least 100 μM begins a process of tenderization by degrading or disassociating many of the structural and bonding proteins of the muscle (Takahashi, 1996). This theory is based specifically on five different ways in which calcium plays a direct role in the tenderization of muscle proteins.

First of these is that calcium binds to phospholipids in Z-discs. These phospholipids have an appreciable affinity for calcium. This binding causes a freeing of the newly formed calcium-phospholipid compound, which weakens the structure of the Z-disc (Shimada & Takahashi, 2003). This supports the fact that Z-disks weaken with postmortem aging, and that upon homogenization, aged meat seperates into 1-4 sarcomere units (Takahashi, 1996).

Second is that the calcium ions cause a disassociation of a novel protein paratropomysosin, which has been found to modify the actin-myosin interaction (Nakamura & Takashi, 1985). Although this paratropomyosin is uniquely located at the A-I band junction, an increase in calcium to 100 μM causes the movement of paratropomyosin to the thin filament, where its affinity for myosin binding sites on actin cause it to accelerate the rate of disassociation of actin and myosin (Takahashi, 1996).

Third, titin, a structural protein that constitutes a third filament in the sarcomere from the M line to the Z disk (Aberle et al., 2001), has been found to be severed at varying concentrations of calcium (Tatsumi & Takahashi, 2003). This severance begins at concentrations of 10 μM and peaks at concentrations of 100 μM (Tatsumi & Takahashi, 2003), significantly less than the concentration of 970 μM of sarcoplasmic calcium in bovine *longissimus* found by Parrish and others (1981).

Fourth, nebulin filaments, which extends as a structural protein from the A band to the Z disk of the sarcomere and serves to anchor the thin filament to the Z disk (Aberle et al., 2001), are severed by the binding of calcium ions (Tatsumi & Takahashi,

2003). Much like titin, nebulin severance begins at concentrations of 10 μM and reaches a maximum at 100 μM of calcium (Tatsumi & Takahashi, 2003).

Finally, Ji and Takahashi (2006) cited Takahashi (1999) as stating that desmin is broken down rapidly by the binding of calcium ions. Desmin functions as a structural protein specifically associated with the exterior of the Z disk (Aberle et al., 2001). Because of desmin's function it is classified as a cytoskelatal protein (Takahashi, 1996).

Takahishi (1996) also postulated that calcium may have a role in the weakening of the perimysial and endomysial connective tissues during post mortem aging of beef. Yet this is solely a theory, Takahashi (1996) stated that "The mechanism responsible for the degradation of proteoglycans during postmortem ageing of meat is unknown….. It seems reasonable to assume that the structural weakening of the endomysium and perimysium is induced by 0.1 mM calcium ions under non-physiological conditions, as occurs in the myofibrillar structures." This remains to be shown.

One other theory currently on calcium's role in the tenderizing of beef is the calcium driven enzymatic theory, which states that the postmortem release of calcium from the sarcoplasmic reticulum activates μ calpain, which acts as a protease in the degradation of structural proteins of the muscle fibers. The calcium release also activates calpastatin, which inhibits the activity of μ calpain (Koohmaraie, 1988; Koohmaraie et al., 1988a; Koohmaraie et al., 1992; Koohmaraie et al., 1988b).

Thus, calcium concentration and collagen characteristics have been correlated to tenderness in beef in the past. The research reviewed has resulted in varying levels of

correlation; therefore, it becomes necessary to further study these systems in order to further understand their roles on tenderness.

CHAPTER III

MATERIALS AND METHODS

Steak selection

Data from previous work (Metteaur, 2008; Nicholson, 2008) on F_2 Nellore x Angus cross cattle were used to select families that had high Warner-Bratzler shear values, and families with markedly low Warner-Bratzler shear values. Families differing in tenderness were selected as a subset of this large population in order to create a smaller population divergent in tenderness. The steaks from these families then were labeled as "tough" (n = 28 steaks) or "tender" (n = 10 steaks) using a Warner-Bratzler shear force value of the non-electrically stimulated steak of 30 N as the line between. A total of 19 animals were selected from these families.

Electrical stimulation

The animals were harvested at the Rosenthal Meat Science and Technology Center at Texas A&M University. The carcasses were stimulated by side on the rail with the right side always being stimulated and the left not stimulated as a control. A single electrical probe was inserted into the carcass between the thoracic vertebrae and the scapula with the rail acting as the ground. Each right side received 525 volts (AC), 2 amps, 2 seconds on, 2 seconds off, for 15 impulses. Electrical stimulation was applied within 1 h postmortem. After harvest the carcasses were placed in a blast-chill cooler (1-2º C) for 24 h then in a holding cooler (2-4º C) for 24 h (Metteaur, 2008; Nicholson, 2008).

pH, marbling, and steak preparation

Muscle pH was measured in intervals at 3 and 24 h postmortem. Muscle pH was measured from the *m. longissimus lumborum*, in the caudal half, using an IQ pH/temperature instrument (model IQ150, probe pH 57-SS, IQ Scientific Instruments, Inc., Carlsbad, CA). At 48 h postmortem, the carcasses were ribbed between the 12th and 13th rib, and allowed to bloom for 15 min. Marbling was determined by trained Texas A&M University personnel using the USDA (1997) quality grade standards.

Loin steaks were removed 48 h postmortem, after marbling data were obtained. The cranial half of the *m. longissimus lumborum* was removed and the strip loin section was cut into 2.54 cm steaks beginning from the cranial end. The first steak from each side was assigned to Warner-Bratzler shear force, the second to sensory (data not presented), the third was utilized for calcium concentration and collagen characteristics, the fourth to sarcomere length, and the fifth to MFI. After cutting, the steaks were trimmed of external fat and vacuum packaged, and stored at -23.33º C (Metteaur, 2008; Nicholson, 2008) .

Warner-Bratzler shear force

Loin steaks assigned for Warner-Bratzler shear force determinationss were cooked to an internal temperature of 70 °C using Farberware Open Hearth broilers (Farberware Company, Bronx, NY). Internal temperature was monitored continually using type-K thermocouples (model KTSS-HH, Omega Engineering, Inc., Stamford CT), inserted into the geometric center of each steak, and attached to a Thermocouple Input Benchtop Meter (model BS 6001A, Omega Engineering, Inc., Stamford, CT).

The cooked steaks were covered and chilled overnight, after which six 1.27 cm core samples were taken from each steak, with cores taken parallel to the muscle fiber orientation. Each core was sheared on a Universal Testing Machine (model 5STM-500, United Calibration Corp., Huntington Beach, CA) equipped with a V-notch Warner-Bratzler blade, and a 20 kg compression load cell with a cross-head speed of 200 mm/min. The peak force (N) needed to shear each core was recorded (Metteaur, 2008; Nicholson, 2008) .

S*arcomere length*

Sarcomere length was measured using the procedure describe by Cross, West and Dutson (1981). A Spectra-Physics model 155SL helium-neon laser (0.95mW, λ= 0.6328) was pre-warmed before taking measurements. Three to five grams of minced loin muscle tissue was removed from each steak set apart for sarcomere length determination. The sample was homogenized in 15-20 mL of cold (4° C) buffer solution (85.58 g, 0.25 M sucrose, 0.15 g, 0.02 mM KCl, and 1.4 g, 0.005 M iodoacetate; adjusted to pH 7.0 and brought to 1 liter volume with distilled water) at a low speed for 10-15 sec. One drop of the homogentate was place on a glass microscope slide, and a cover slip was placed over the homogenate. The slide then was placed on the laser stage, with the distance from the top of the slide to the baseboard of the laser stand being set at 100 mm. The distance from the origin and the first order diffraction band was recorded (Metteaur, 2008; Nicholson, 2008). The length of the sarcomere was calculated in μm using the formula of Cross et al. (1981).

Myofibrillar fragmentation index

Myofibrillar fragmentation index was determined using the procedure of Olson, Parrish and Stromer (1976) and revised by Culler, Parrish, Smith and Cross (1978). Minced muscle (4 g) was added to a Eberbach blender with 40 mL of isolating medium (100 mM KCl, 20 mM KPO4 (pH 7.0), 1 mM EDTA, 1 mM MgCl2, and 1 mM sodium azide) and blended for 30 sec. The homogenate was centrifuged at 1000 x *g* for 15 min, and the supernate was decanted. The pellet was resuspended in 40 mL of isolating medium using a glass stir rod, then centrifuged again at 1000 x *g* for 15 min and the supernate was decanted. The pellet was resuspended in 10 mL of isolating medium using a glass stir rod and passed through a polyethylene strainer to remove connective tissue and debris, an additional 10 mL of isolating medium was used to facilitate passage of myofibrils through the strainer. The protein assay was conducted by placing 0.25 mL of each suspension into 13 × 100 mm glass cuvette, along with 0.75 mL isolating medium and 4 mL biuret reagent; this was vortexed. The sample then was set in a dark room for 30 min while standards from Bovine Serum Albumin (BSA) were run to establish a standard curve. Absorbance was read at 540 nm with a Bausch and Lomb Spectronic 20 colorimeter with a large split width, absorbance was multiplied by 200 to give the myofibril fragmentation index (Metteaur, 2008; Nicholson, 2008).

Collagen content and solubility

The steaks that were to be utilized for calcium concentration and collagen characteristics determination were denuded and cut in half. One half was minced and frozen in liquid nitrogen and powdered in a blender (Waring, Torrington, CA) for

measuring collagen content and solubility. The other half was minced for measuring calcium concentration.

Duplicate samples (4.0-4.1g) from each powdered steak were taken, with the exact weight being recorded. The samples were placed in 50-ml disposable centrifuge test tubes. To each tube 12 ml of one-quarter strength Ringer's solution (250 ml Ringer's stock, 7.0g NaCl, 0.026g $CaCl_2$, 0.35g KCl dissolve in 1 liter of ddH_2O) brought to volume in a 1 L volumetric flask with ddH_2O, was added. Each tube then was stirred exactly 20 times, with a separate glass rod for each sample, leaving the glass rod in the tube. The tubes then were placed in a water bath at 78ºC for 60 min. Every 5 minutes each tube was stirred exactly 10 times. The glass rods were removed and the tubes were capped then cooled in a 4ºC cooler for 15 min.

After cooling, the tubes were centrifuged for 20 min at 27,216 x *g* at 2ºC using a JA-17 (Beckman Coulter, Fullerton, CA) rotor in an Avanti J-25 centrifuge (Beckman Coulter, Fullerton, CA). During centrifugation, cooking jars were labeled with a jar for the residual and a jar for the supernate of each sample. After centrifugation, the supernate was decanted into a labeled jar. Eight milliliters of one-quarter strength Ringer's solution was added back to the residual and stirred 10 times, using a separate glass rod for each sample. The samples then were centrifuged again for 20 min at 27,216 x *g* at 2ºC. Again the supernate was decanted off into the same supernatant marked jars. Five milliliters of ddH_2O was added to the residual meat pellet and mixed well. This was then poured into the cooking jar label for the residual of each sample.

Another 5 ml of ddH_2O was used to rinse the remaining pellet out of the test tube and this was added to the residual jar.

Thirty milliliters of 7 N sulfuric acid, which is made by adding 375 ml H_2SO_4 slowly to 750 ml ddH_2O, in a 2 L volumetric flask in an ice bath, then brought to volume and cooled to room temperature, was added to each of the combined supernates. Twenty milliliters of the 7 N sulfuric acid was added to each of the residual jars. The jars were sealed and placed in an oven under a hood, where they were cooked at 105 ºC for 16 h. When the 16 h had elapsed, the jars were removed from the oven and lids were immediately removed, the samples then were left under the hood to cool and vapors were allowed to escape.

After cooling, the samples were transferred to volumetric flasks, the supernate hydrolysates were transferred to 200-ml flasks and 500-ml flasks for the residual samples. The cooking jars were rinsed with ddH_2O and that was added to the flasks, the flasks were then brought to volume, of 200 ml for supernate and 500 ml for residual, with ddH_2O. Each sample then was filtered through #2 filter paper into 50-ml test tubes, collecting at least 50 ml. The remaining liquid was discarded.

A hydroxyproline stock solution was made using trans-4-hydroxy-L-proline (Sigma-Aldrich, St Louis, MO) by dissolving 0.025 g of the hydroxyproline powder into 250 ml of 0.001 N HCl. Five standard solutions were made using this stock solution: the first with 2 ml hydroxyproline stock solution and 98 ml ddH_2O. The next with 4 ml hydroxyproline stock solution and 96 ml ddH_2O, followed by a mixture of 6 ml stock solution and 94 ml ddH_2O, with the next standard by made by combing 8 ml stock

solution and 92 ml ddH_2O, and finally 10 ml stock solution and 90 ml ddH_2O, these five dilutions were the standards for the color curve.

Two milliliters of each standard solution were added to duplicate test tubes, along with two blank tubes with 2 ml ddH_2O. Also 2 ml of each of the filtrates were added to individual test tubes. To all tubes, including standards and blanks, 1 ml of an oxidant solution, 1.41 g chloramine T (Fisher Scientific, Pittsburgh, PA) dissolved in 100 ml of buffer solution, 30.0 g citric acid monohydrate, 15.0 g NaOH, 90.0 g sodium acetate trihydrate, dissolved in 500 ml ddH_2O then put in a 1 liter volumetric flask to which 290 ml isopropanol was added. Then pH was adjusted to 6.0 with NaOH or acetic acid, was added. The tubes were vortexed and then rested for exactly 20 minutes, at room temperature.

After the tubes had rested for 20 min, 1.0 ml of the color reagent was added. The color reagent was made by dissolving 10g 4-dimethylbenzaldehyde (Fisher Scientific, Pittsburgh, PA) in 35 ml 60% perchloric acid. Sixty percent perchloric acid was made by adding 50 ml ddH_2O to 428.33 ml of 70% perchloric acid (Sigma-Aldrich, St Louis, MO) in a 500-ml volumetric flask, allowing it to cool and then bring to volume. Sixty five ml isopropanol was added slowly to the mixture of 4-dimethybenzaldehyde and 60% perchloric acid. The tubes then were vortexed and were covered with aluminum foil and placed in a 60ºC hot water bath for 15 min. After the 15 min, the tubes were removed from the hot water bath, uncovered, and allowed to cool to room temperature. Once cooled, the samples were transferred to cuvets and read using a UV-Visible Spectrophotometer (Varian Inc., Palo Alto, CA) at 558 nm. The blanks and standards

were read first and the readings recorded. The color curve was fit using JMP 8.0 (SAS Institute, Cary, NC).

Using Microsoft Excel 2007 (Microsoft Corp., Redman, WA), the readings were fit to the standard curve to give the mg of hydroxyproline in each sample. The hydroxyproline amount was multiplied by the dilution factors (200 for the supernate and 500 for the residual), and divided by the initial sample weight of each individual sample, this was the total hydroxyproline amount (mg).

To determine the actual collagen amount the hydroxyproline amount was multiplied by 7.52 for the supernate and 7.25 for the residual (Cross et al., 1973), and then divided by 1,000 to give supernate and residual collagen amounts in mg/g. The supernate was the soluble collagen and the residual contained insoluble collagen. Total collagen (mg collagen/g meat), was defined as soluble collagen plus insoluble collagen and percent solubility was calculated as soluble collagen divided by total collagen multiplied by 100. These methods are derived from Cross et al. (1973) who derived their methods, with some minor adjustments, from Woessner (1961) and Hill (1966).

Calcium concentration

Five grams of each minced steak was homogenized seperately with 25 ml of ddH_2O in a blender (Waring, Torrington, CA), and placed in labeled 50-ml centrifuge test tube. The homogenate was centrifuged at 37,000 x g_m for 3 h in an Avanti J-25 centrifuged (Beckman Coulter, Fullerton, CA). During centrifugation 15-ml test tubes were labeled in duplicate for each sample. Two milliliters of the supernate from each

sample was removed and placed in the pre-labeled 15-ml test tubes. The remaining liquid and pellets were discarded.

The supernate then was treated with 4 ml of 5% TCA in order to precipitate out the remaining protein matrix in the supernate. Ten minutes later 4 ml of 0.5% strontium chloride (Sigma-Aldrich, St Louis, MO) was added to act as ionic interference (Ji & Takahashi, 2006; Parrish et al., 1973). The mixture then was centrifuged again at 1700 g_{max} for 10 minutes to spin out the precipitated protein.

Nine milliliters of the supernatant from this final centrifugation was then removed and placed in 50-ml vials. The vials were used to transfer the liquid to the flame atomic absorption spectrophotometer (Perkin Elmer, Waltham, MA) where samples were read against standards of 2, 4 and 6 ppm calcium that had been treated with the same compounds as the samples. This procedure was taken from Ji and Takahashi (2006), who modified the Parrish et al. (1981) methodology, with minor adjustments.

The standards were prepared by first weighing out exactly 0.312 g of $CaCO_3$. The $CaCO_3$ then was transferred to a 250-ml beaker, 25 ml of ddH_2O was added as well as 12 drops of 12 M HCl. The beaker was covered with a watch glass while the reaction took place and the $CaCO_3$ dissolved. After the $CaCO_3$ had dissolved, the walls of the beaker and the bottom of the watch glass were rinsed with ddH_2O to ensure that all of the calcium remained in the liquid. The beaker then was transferred to a heating plate and heated just until boiling. After heating 50 ml of ddH_2O was added to the beaker and mixed. The entire solution then was transfered to a 250-ml volumetric flask and the

beaker was rinsed three times with ddH_2O. This was also transferred to the flask. The solution then was brought to volume in the volumetric flask, the flask was stopped and inverted three times; this was a 500 ppm solution. Ten milliliters of this solution was transferred to a 100-ml volumetric flask, and brought to volume with ddH_2O, then stopped and inverted to make a 50 ppm solution. Three separate 100-ml volumetric flasks were labeled one with 2 ppm, one with 4 ppm and the last with 6 ppm. To the 2 ppm flask, 4 ml of the 50 ppm solution was added and then the flask was brought to volume, stopped and inverted to mix. To the 4 ppm flask, 8 ml of the 50 ppm solution was added, the flask was brought to volume and then mixed in the same fashion (Terezakis, 2002). To the 6 ppm flask, 12 ml of the 50 ppm solution was added, then the flask was brought to volume, this was also mixed in like fashion. This provided standards of 2 ppm Ca, 4 ppm Ca, and 6 ppm Ca. These standards were tested against purchased lab grade calcium standard stock solution, and were found to be of equal value.

Data analysis

Analysis of variance was performed using the Generalized Linear Model of JMP 8.0 (SAS Institute, Cary, NC). The main effects were, Tenderness value, Treatment and Tenderness value x Treatment, with an $\alpha < 0.05$. Treatment being electrically stimulated or non-electrically stimulated and tenderness values being greater than or equal to 30.00 N Warner-Bratzler shear on the non-stimulated loin steaks, and less than 30.00 N Warner-Bratzler shear on the the non-stimulated loin steaks. Least squares means were calculated for calcium concentrations, total collagen, percent soluble collagen,

sarcomere length, myofibrillar fragmentation index, marbling, Warner-Bratzler shear force, 3 h postmortem pH and 24 h postmortem pH by tenderness value and by treatment and if differences where found in the effect from the analysis of variance table, least squares means where separated using the pdiff function of JMP 8.0 (SAS Institute, Cary, NC). Where interactions occur bar graphs were used to illustrate interactions. Simple correlations of all variables were also run. The effects of electrical stimulation, sarcomere length, marbling, myofibrilar fragmentation index, total collagen content, collagen solubility, sarcoplasmic free calcium concentration, 3 h postmortem pH and 24 h postmortem pH on Warner-Bratzler shear values were determined using the stepwise function of the JMP 8.0 program (SAS Institute, Cary, NC).

CHAPTER VI

RESULTS AND DISCUSSION

Analysis of variance for all variables can be found in Appendix A. Table 1 presents the least squares means for the variables by the main effects of tenderness value and treatment, along with the P-values from the analysis of variance. To obtain the tenderness values an arbitrary line was set at 30 N to separate "tougher" and "more tender" steaks in order to categorize the data for analysis. The steaks category was set based on the non-electrically stimulated steaks Warner-Bratzler shear values and applied to the electrically stimulated steaks. Table 1 illustrates the least squares means for calcium concentration, total collagen, soluble collagen, and sarcomere length.

Calcium concentration was shown to differ significantly by treatment (P = 0.0027). Yet no significant differences were found for calcium concentration by tenderness value. According to Hwang, Devine and Hopkins (2003), this is a result from the acceleration of pH decline by electrical stimulation. This pH decline is "mirrored" by increase in "free" calcium concentration. Thus, at like temperatures, muscles that have been electrically stimulated will demonstrate higher "free" calcium concentrations.

No statistical differences were found for total collagen content either by treatment or tenderness value, these values were comparable to those found in *longissimus lumborum* steaks by Hagar (2000), as well as the collagen content values found by Whipple et al. (1990). Neither were there any significant differences for collagen solubility either by treatment or tenderness value, these values were slightly

Table 1
Least squares means for calcium concentration, total collagen, % soluble collagen and sarcomere length.

Main Effects	Calcium concentration, μM	Total collagen, mg/g	Soluble collagen, %	Sarcomere length, μm
Treatment [a]				
ES	780.58a	2.65	14.03	1.77
NON-ES	429.55b	2.50	12.38	1.73
P > F	0.0027	0.8556	0.1674	0.3383
Tenderness value				
< 30 N	627.30	2.54	13.31	1.78
≥ 30 N	582.83	2.59	13.10	1.72
P > F	0.6842	0.8939	0.8556	0.2425
RMSE	294.221	0.633	2.875	0.127

[a] One side was electrically stimulated (ES); the other side was not electrically stimulated (NON-ES)
Within a column by main effect, means lacking a common letter (a-c) differ ($P < 0.05$)

higher than those found by Hagar (2000) and Cross and others (1973), but still within the solubility range reported by Goll et al. (1964) and by Whipple et al. (1990). It should be noted that the differences may be a result of the difference in collagen solubility between *Bos indicus* and *Bos taurus* influenced cattle (Whipple et al., 1990) as well as updates to the procedure that was used by Cross et al. (1973), which include stirring during cooking of the meat in the hot water bath. It is interesting to note that there is a 1.56 percentage point difference in the amount of soluble collagen, between the electrically stimulated and non-electrically stimulated sides; although this is not significant there is a possibility of some sort of interaction between treatment and calcium concentration, as the *p-value* is approaching the limit of 0.05.

No statistical differences were found in sarcomere length by treatment or by tenderness value. This is concurrent with the data reported by Metteaur (2008), as well as work done by Savell, Smith, Dutson, Carpenter and Suter (1977); however, this does not mesh with Taylor (2003), when he states that "The structural changes (from electrical stimulation) include stretching of sarcomeres…" Although there may be some stretching during electrical stimulation it does not appear to be statistically significant. What may be happening is that in the past the non-electrically stimulated sides are undergoing cold shortening, and this is responsible for the sarcomere length differences that have been seen between electrically-stimulated and non-stimulated sides, that does not appear to be the case with these data. Meaning there may not have been any, or minimal, cold shortening on the non-stimulated sides.

Table 2 reports the least squares means for myofibrillar fragmentation index, marbling scores, 3 h postmortem pH values and 24 h postmortem pH values. There were no significant differences between the myofibrillar fragmentation index values by treatment. This is contradictory to the data reported by Metteaur (2008), but is in line with the data reported by Sonaiya, Stouffer and Beerman (1982), although they also reported that myofibrillar fragmentation index values were consitantly higher for the stimulated carcasses, they were not statistically significant. There were also no significant differences for myofibrillar fragmentation index values by tenderness value. This is again contradictory to the data reported by Metteaur (2008). This is possibly indicative of the difference in the formation of this model as well as the smaller sample size and different main effects.

There were no significant differences in marbling either by treatment or by tenderness values. This is in line with the data presented by Sonaiya et al. (1982), where they show no significant difference between marbling scores of electrically stimulated and non-electrically stimulated beef, as well as data reported by Savell, Smith and Carpenter (1978).

There was a significant difference ($P < 0.0001$) on 3 h postmortem pH value between treatments. This matches data that was reported by Metteaur (2008), as well as information presented by Hwang et al. (2003) and data presented by Shaw and Walker (1977). This rapid decline in pH as a result of electrical stimulation is thought to play a very significant role in the significant difference in calcium concentrations between

Table 2
Least squares means for MFI, marbling, 3 h pH and 24 h pH.

Main Effects	Myofibrillar fragmentation index	Marbling[b]	3 hour pH	24 hour pH
Treatment[a]				
ES	46.30	452.36	5.42b	5.60
NON-ES	45.49	453.00	5.99a	5.62
P > F	0.9544	0.9898	<0.0001	0.4971
Tenderness value				
< 30 N	42.75	460.00	5.68	5.61
≥ 30 N	49.05	445.35	5.72	5.62
P > F	0.6569	0.7711	0.6868	0.8023
RMSE	38.150	135.509	0.247	0.105

[a] One side was electrically stimulated (ES); the other side was not electrically stimulated (NON-ES)
[b] Moderately Abundant = 800-899, Slightly Abundant = 700-799, Moderate = 600-699, Modest = 500-599, Small = 400-499, Slight = 300-399, Traces = 200-299, and Practically Devoid = 100-199.
Within a column by main effect, means lacking a common letter (a-c) differ ($P < 0.05$)

electrically stimulated and non-electrically stimulated steaks (Hwang et al., 2003). There was no significant difference on 3 h postmortem pH between tenderness values.

There were also no significant differences found on 24 h postmortem pH by treatment. This is not in line with the information presented by Metteaur (2008), who showed a significant difference by treatment, reported as side, ($P = 0.0012$). This difference is undoubtedly a result of having only a subset of the steaks used by Metteaur, in the presented research. This seems to indicate that the electrically stimulated steaks used in this model experience a more rapid decline in pH than the non-electrically stimulated steaks, but both were normalized to a pH of roughly 5.6 by the time 24 h had elapsed. There were also no significant differences on 24 h postmortem pH by tenderness value.

Figure 1 illustrates the interactive effect of tenderness value and treatment on Warner-Bratzler shear force values ($P = 0.0004$). This interaction shows that for the steaks with non-electrically stimulated shear force values $\geq$ 30 N, there was a significant difference in Warner-Bratzler shear force values between the electrically stimulated and the non-electrically stimulated steaks. There was not, however, a difference in Warner-Bratzler shear force values between the electrically and non-electrically stimulated steaks of the < 30 N non-electrically stimulated Warner-Bratzler shear force value steaks.

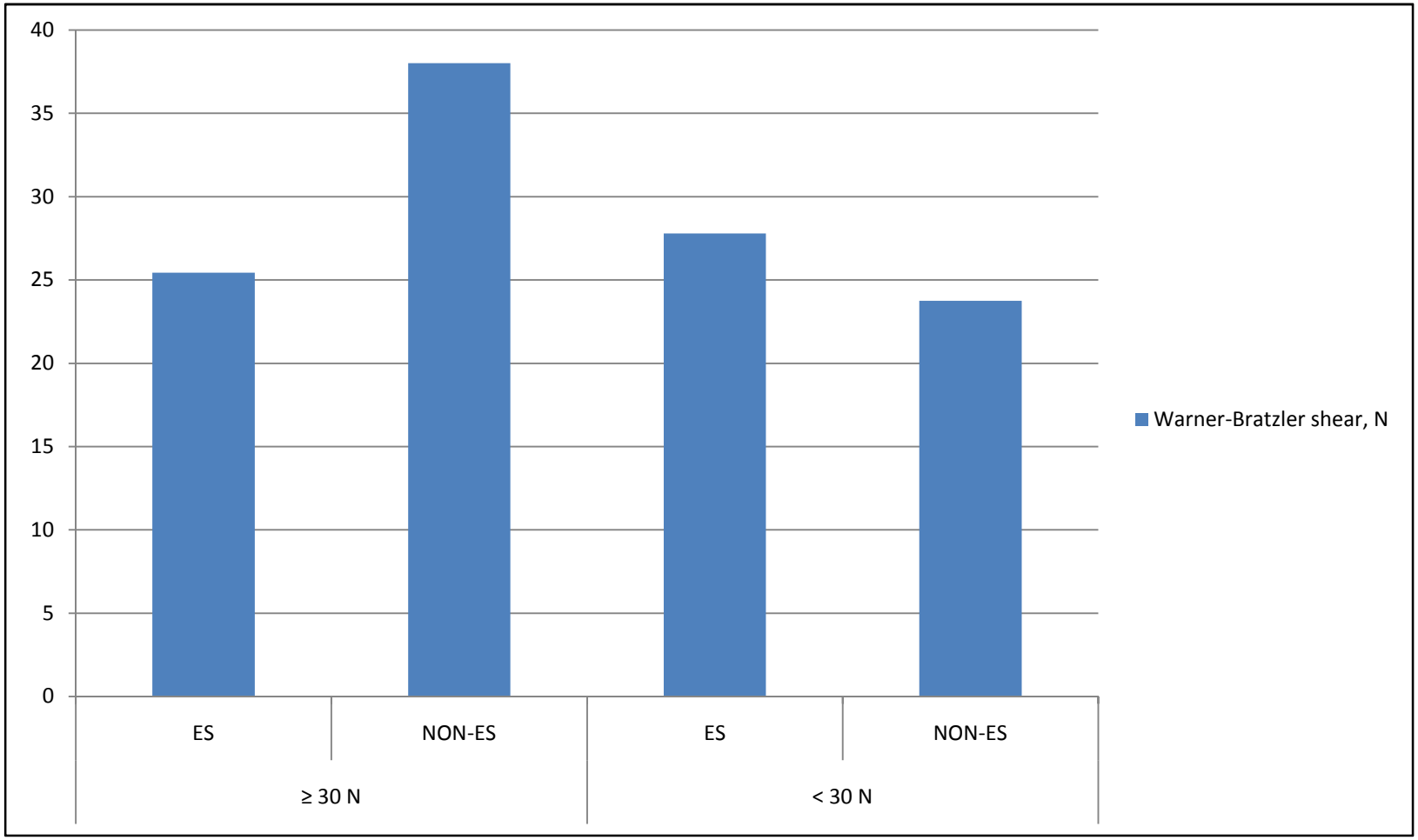

Figure 1
Warner- Bratzler shear, N for tenderness value as stratified by treatment

Table 3 shows the simple correlations between the variables included in the model. Table 3 specifically contains the correlations of calcium concentration, total collagen, soluble collagen, marbling values, sarcomere length, myofibrillar fragmentation index, Warner-Bratzler shear force values, 3 h postmortem pH and 24 h postmortem pH. Tenderness value and treatment were not included because they are simply arbitrary values used to describe an action taken on the steaks. Complete pairwise correlations are located in Appendix A.

Calcium concentration had a negative correlation to Warner-Bratzler shear force ($P = 0.0478$). Which has been specifically tested in the past, this interaction has been postulated by Takahashi (1996), who stated that it was likely that the degradation of structural proteins of the muscle fiber occurs in the concentration of at least 100 μM of free calcium ions in the sarcoplasm. There is also a negative correlation ($P = 0.0114$) between calcium concentration and 3 h postmortem pH of the *m. longissimus lumborum*. This is a strengthening point to the argument made by Hwang et al. (2003), as described in a previous section of this document. It is interesting to note that although there is not a significant correlation between calcium concentration and 24 h postmortem pH, it is approaching significance ($P = 0.0521$). This also would support Hwang et al. (2003), and may only not show significance due to the smaller sample size in this model. It is also interesting to note that there appears not to be a significant correlation between calcium concentration and collagen solubility. While this is clearly true in this model, Takahashi (1996) postulates that there is possibly a degradation of proteoglycans by calcium at concentrations of 100 μM or greater, in a similar manner to the degradation of

Table 3
Combined simple correlations of variables[a]

	Total collagen, mg/g	Soluble collagen, %	Marbling[b]	Sarcomere length, μm	Myofibrillar fragmentation index	Warner-Bratzler shear, N	3 hour pH	24 hour pH
Calcium concentration, μM	-0.05	0.12	0.22	0.21	-0.01	**-0.32**	**-0.41**	-0.32
Total collagen, mg/g		**-0.80**	**-0.42**	0.02	0.01	-0.08	-0.03	0.01
Soluble collagen, %			0.26	-0.09	-0.02	0.01	-0.19	-0.04
Marbling [b]				0.20	-0.25	-0.07	-0.16	-0.02
Sarcomere length, μm					-0.03	-0.11	-0.20	-0.14
Myofibrillar fragmentation index						0.04	-0.14	**-0.44**
Warner-Bratzler shear, N							**0.65**	0.13
3 hour pH								**0.38**

[a] Values in bold are significant at $P < 0.05$
[b] Moderately Abundant = 800-899, Slightly Abundant= 700-799, Moderate = 600-699, Modest = 500-599,Small = 400-499, Slight = 300-399, Traces = 200-299, and Practically Devoid = 100-199

structural proteins mentioned previously. It may be possible that there is an increased solubility of collagen by calcium, but that it is a threshold system. That is at 100 μM of sarcoplasmic free calcium optimal increased solubility of collagen occurs, which would be easily met at the levels recorded in Table 1. Again further research is needed to explore the effect of calcium on collagen solubility.

Total collagen has a strong negative correlation (P <0.0001) to collagen solubility. This is in line with the data presented by Torrescano, Sánchez-Escalante, Giménez, Roncalés and Beltrán (2003) who showed a high positive correlation of total collagen to insoluble collagen, so the inverse would be expected for soluble collagen. Total collagen does not increase with age, however solubility does decrease (Cross et al., 1973) when there is more collagen there is more opportunity for increased cross-linking and inherently decreased solubility, this may be one explanation to the negative correlation between total collagen and collagen solubility. Total collagen is also negatively correlated (P = 0.0143) to marbling. The non-significant correlation between total collagen and Warner-Bratzler shear force is similar to the data reported by Cross et al. (1973).

Myofibrillar fragmentation index values had a negative correlation (P = 0.0058) to 24 h postmortem pH values. This is especially interesting because of the strength of the correlation, yet myofibrillar fragmentation does not significantly correlate to 3 h postmortem pH, nor to any other variables. Which is unique in that one would expect a variable that would correlate with both pH values or neither value. It is possible that the

increase of myofibrillar fragmentation during aging (Silva, Patarata & Martins, 1999) is actually a result of pH. More research is needed to understand this process more fully.

Warner-Bratzler shear force values are positively correlated ($P = 0.0103$) to 3 h postmortem pH values. This is unique because there is not a significant correlation between Warner-Bratzler shear force values and 24 h postmortem pH values. Although this seems to be contradictory to the data presented by Yu and Lee (1986), it is actually in accordance with the data that they present. Their data showed that at pH 5.8 to 6.3, which they label as intermediate pH steaks, an increase in Warner-Bratzler shear force values from steaks with pH less that 5.8 and steaks with pH greater than 6.3 occurs, which are labeled as low and high pH steaks, respectively. The mean 3 h postmortem pH values for the steaks in this model are on the high side of low and spilling over into the low end of intermediate pH steaks. It would thus be logical that an increase in pH would cause an increase in Warner-Bratzler shear force values in this model, but once the steaks reach a pH of 6.3 or greater the correlation changes, according to Yu and Lee (1986), and becomes negative.

This work seems to be in line with the data that Shackelford, Koohmaraie and Savell (1994) cited from Marshall and Tatum (1991) who reported that pH below steaks below 3 h pH of 5.8 generally were the most tender. Marshall and Tatum (1991) also showed that 3 h pH was not highly correlated with tenderness. Shackelford et al. (1994) state that 3 h postmortem pH is not an accurate predictor of tenderness.

It is not hard, however, to understand why there is not a statistical correlation between Warner-Bratzler shear force values and 24 hour postmortem pH values, as the variation in 24 hour postmortem pH was limited.

There is a positive correlation ($P = 0.0176$) between 24 h postmortem pH values and 3 h postmortem pH values. This seems to be a logical correlation in that higher initial pH values tend to result in higher ultimate pH values.

Tables 4 and 5 report the simple correlations of variables for the ES sides and NON-ES sides respectively. When the separation between the treatments is made many of the correlations seen in table 3 disappear. The correlations that remain on the ES sides are total collagen to soluble collagen and total collagen to marbling. Myofibrillar fragmentation index and 24 h postmortem pH are correlated as well. Also 3 h and 24 h postmortem pH are still correlated. For the NON-ES side only total collagen and soluble collagen are correlated.

The loss of some of the correlations when the data is separated by treatment may be indicative of the effect that treatment plays on some of the variables. For example both 3 h postmortem pH and Warner-Bratzler shear force were correlated in the Table 3 with calcium concentration but they are not correlated when separated by treatment, suggesting that the correlation may have been a result of the effect of treatment more than the effect of the variables. It could also, however, signify the means by which tenderization occurs in electrically stimulated beef.

Table 4
Simple correlations of variables of the ES side[a]

	Total collagen, mg/g	Soluble collagen, %	Marbling[b]	Sarcomere length, μm	Myofibrillar fragmentation index	Warner-Bratzler shear, N	3 hour pH	24 hour pH
Calcium concentration, μM	-0.21	0.09	0.31	0.15	0.00	-0.20	-0.09	-0.24
Total collagen, mg/g		**-0.81**	**-0.47**	-0.08	0.09	-0.03	0.15	0.19
Soluble collagen, %			0.15	0.14	0.03	0.37	-0.09	-0.21
Marbling [b]				0.07	-0.19	-0.38	-0.35	0.02
Sarcomere length, μm					-0.09	-0.03	0.30	-0.18
Myofibrillar fragmentation index						0.21	-0.31	**-0.47**
Warner-Bratzler shear, N							0.07	-0.06
3 hour pH								**0.67**

[a] Values in bold are significant at $P < 0.05$
[b] Moderately Abundant = 800-899, Slightly Abundant= 700-799, Moderate = 600-699, Modest = 500-599,Small = 400-499, Slight = 300-399, Traces = 200-299, and Practically Devoid = 100-199

Table 5
Simple correlations of variables of the NON-ES side[a]

	Total collagen, mg/g	Soluble collagen, %	Marbling[b]	Sarcomere length, μm	Myofibrillar fragmentation index	Warner-Bratzler shear, N	3 hour pH	24 hour pH
Calcium concentration, μM	0.23	-0.32	0.21	0.25	-0.02	-0.02	0.09	-0.37
Total collagen, mg/g		**-0.93**	-0.15	0.55	-0.16	-0.03	-0.20	-0.35
Soluble collagen, %			0.26	-0.51	0.11	0.04	0.10	0.29
Marbling [b]				0.17	-0.31	0.06	-0.22	-0.05
Sarcomere length, μm					-0.09	0.07	-0.41	-0.01
Myofibrillar fragmentation index						-0.06	-0.14	-0.43
Warner-Bratzler shear, N							0.03	0.08
3 hour pH								0.15

[a] Values in bold are significant at $P < 0.05$
[b] Moderately Abundant = 800-899, Slightly Abundant= 700-799, Moderate = 600-699, Modest = 500-599,Small = 400-499, Slight = 300-399, Traces = 200-299, and Practically Devoid = 100-199

Tables 6 shows regression equations to predict Warner-Bratzler shear force values for the electrically stimulated steaks. Stepwise regression was used in comparing the effect of the variables on Warner-Bratzler shear force values. When selecting a regression equation it is useful to look at various statistics, those included on this table are in accordance with the suggestions of MacNeil (1983). The statistics are R^2, root mean squared error (RMSE), and Mallows statistic (Cp). According to MacNeil's (1983) recommendations, the most useful regression equation optimizes the R^2 while at the same time minimizing the root mean squared error, and the mallows statistic should be the closest to or below the p, which is the sum of the variables plus 1. This should all be done while minimizing p, ultimately by minimizing the independent variables.

Table 6 shows that the first variable in the electrically stimulated side that is included in the regression equation is marbling ($R^2 = 0.13$), it would thus appear that marbling, in this model, is the single greatest predictor of Warner-Bratzler shear force value. The best two variable equation, however was total collagen and soluble collagen ($R^2 = 0.22$) representing 0.09 increase in the ability to predict Warner-Bratzler shear force value.

Table 6
Stepwise linear regression equations for electrically stimulated steaks to predict 14 d WBS

	Independent variables	Intercept	β-value	R^2	RMSE	Cp
1	Marbling[a]	32.58	-0.01460	0.13	4.06	-2.11
2	Total collagen, mg/g	-6.36	5.3247	0.22	3.96	-1.33
	Soluble collagen, %		1.2965			
3	Marbling[a]	-48.23	-0.0153	0.29	3.93	-0.16
	Soluble collagen, %		0.5475			
	24 hour pH		13.0450			
4	Marbling[a]	-54.38	-0.0165	0.34	3.94	1.19
	Soluble collagen, %		0.5666			
	24 hour pH		15.8442			
	3 hour pH		-1.7005			
5	Marbling[a]	-58.46	-0.0102	0.35	4.08	3.06
	Soluble collagen, %		1.0301			
	Total collagen, mg/g		3.0808			
	24 hour pH		12.2487			
	3 hour pH		-0.4306			
6	Marbling[a]	-62.17	-0.0101	0.36	4.26	5.00
	Soluble collagen, %		1.0265			
	Total collagen, mg/g		3.0283			
	24 hour pH		12.8891			
	3 hour pH		-0.4135			
	MFI[b]		0.0039			
7	Marbling[a]	-59.24	-0.0097	0.36	4.49	7.00
	Soluble collagen, %		1.0277			
	Total collagen, mg/g		3.0435			
	24 hour pH		12.1677			
	3 hour pH		-0.2137			
	MFI[b]		0.0039			
	Calcium concentration, μM		-0.0003			
8	Marbling[a]	-88.70	-0.0146	0.36	4.76	9.00
	Soluble collagen, %		0.6677			
	Total collagen, mg/g		1.3531			
	24 hour pH		25.7850			
	3 hour pH		-6.8266			
	MFI[b]		0.0090			
	Calcium concentration, μM		0.0001			
	Sarcomere length, μm		-0.0717			

[a] Moderately Abundant = 800-899, Slightly Abundant= 700-799, Moderate = 600-699, Modest = 500-599,Small = 400-499, Slight = 300-399, Traces = 200-299, and Practically Devoid = 100-199
[b] Myofibrillar fragmentation index

The best three variable equation ($R^2 = 0.29$) again incorporated marbling, and also used collagen solubility and 24 h postmortem pH value. This equation again increased the R^2, but not by the same amount, a decrease in the RMSE was also reported, and the Cp is at an acceptable level. This equation is an acceptable equation from this table, however it appears that the four variable equation that incorporated marbling, soluble collagen, 24 h postmortem pH and 3 h postmortem pH values was the best equation to predict Warner-Bratzler shear force, as the R^2 (0.34) is nearly peaked compared to the full model ($R^2 = 0.36$). The RMSE is close to the minimum at 3.94, and the Cp is at an acceptable level, all while minimizing the number of variables. Thus, the best prediction equation for predicting Warner-Bratzler shear force values in electrically stimulated steaks was as follows WBS = -58.38 - 0.0165 x (Marbling) + 0.5666 x (Soluble collagen) + 15.8442 x (24 hour pH) - 1.7005 x (3 hour pH).

Table 7 illustrates the prediction equations for non-electrically stimulated steaks, it interesting to note that the R^2 values for these equations are notably smaller than the R^2 values from tables 6 and 8. This is undoubtedly from the greater range in Warner-Bratzler shear force values, which can be seen by the drastically higher RMSE in table 7 over tables 6 and 8.

The single best predictor ($R^2 = 0.05$) for Warner-Bratzler shear force value in non-electrically stimulated steaks in 24 hour postmortem pH values, however this R^2 value is so small as to nullify the validity of this variable as a sole predictor. This seems to be very similar to the variability in tenderness that Blumer (1963) explained using marbling (5%), Blumer's (1963) information was most likely on non-electrically stimulated beef. The best two variable prediction equation ($R^2 = 0.08$) was 24 hour postmortem pH value and marbling score. Again this is a very small R^2 and has limited ability to predict Warner-Bratzler shear force values.

The best three variable prediction equation ($R^2 = 0.10$) included 24 hour postmortem pH, marbling and total collagen content. This is arguably the best of these prediction equations with a R^2 that is not much different from the full model ($R^2 = 0.13$), as well as a minimized RMSE (8.93) and an acceptable Cp (-0.81). Thus, the best prediction equation for predicting Warner-Bratzler shear force values of non-electrically stimulated steaks was WBS = 144.44 - 20.3799 x (24 hour pH) + 0.0107 x (marbling) - 0.2362 x (total collagen). This equation is notably weak in it's predictability of Warner-Bratzler shear force values, but represents the optimal for this model.

Table 7
Stepwise linear regression equations for non-electrically stimulated steaks to predict 14 d WBS

	Independent variables	Intercept	β-value	R^2	RMSE	Cp
1	24 hour pH	-1.87	6.4156	0.05	8.47	-4.41
2	24 hour pH	-5.40	6.6774	0.08	8.64	-2.68
	Marbling[a]		0.0045			
3	24 hour pH	144.44	-20.3799	0.10	8.93	-0.81
	Marbling[a]		0.0107			
	Total collagen, mg/g		-0.2362			
4	24 hour pH	-15.00	4.4393	0.11	9.32	1.14
	Marbling[a]		0.0021			
	Sarcomere length, µm		14.3134			
	MFI[b]		-0.0184			
5	24 hour pH	-1.75	1.8111	0.12	9.77	3.06
	Marbling[a]		0.0025			
	Sarcomere length, µm		16.1888			
	MFI[b]		-0.0222			
	Calcium concentration, µM		-0.0038			
6	24 hour pH	-9.85	1.3828	0.13	10.33	5.03
	Marbling[a]		0.0032			
	Sarcomere length, µm		17.6129			
	MFI[b]		-0.0201			
	Calcium concentration, µM		-0.0044			
	3 hour pH		1.3151			
7	24 hour pH	182.44	-34.5971	0.13	11.03	7.01
	Marbling[a]		0.0111			
	Sarcomere length, µm		16.4264			
	MFI[b]		-0.0364			
	Calcium concentration, µM		-0.0070			
	3 hour pH		2.5621			
	Total collagen, mg/g		1.1197			
8	24 hour pH	168.88	-33.7448	0.13	11.90	9.00
	Marbling[a]		0.0102			
	Sarcomere length, µm		16.5462			
	MFI[b]		-0.0356			
	Calcium concentration, µM		-0.0061			
	3 hour pH		2.5982			
	Total collagen, mg/g		2.5343			
	Soluble collagen, %		0.3810			

[a] Moderately Abundant = 800-899, Slightly Abundant= 700-799, Moderate = 600-699, Modest = 500-599,Small = 400-499, Slight = 300-399, Traces = 200-299, and Practically Devoid = 100-199
[b] Myofibrillar fragmentation index

Table 8 contains the prediction equations for the combined treatments, using treatment as a predictor of tenderness. Incidentally treatment is the single best predictor ($R^2 = 0.36$) of Warner-Bratzler shear force values in this model. This seams to coincide with the data reported in past studies (Savell et al., 1978; Savell et al., 1977). The best two-variable prediction equation ($R^2 = 0.39$) is made up of treatment and sarcomere length.

The best three-variable prection equation ($R^2 = 0.40$) is formed using treatment, total collagen and soluble collagen. This equation is a very strong candidate for the best prediction equation for the combined treatment to predict Warner-Bratzler shear force. The R^2 has seemingly reached a plateau in comparison to the full model ($R^2 = 0.43$), the RMSE is the lowest of any of the prediction equations for the combined treatment regression equations, and the Cp is at an acceptable level with a minimized p. Thus the best prediction equation for predicting Warner-Bratzler shear force value in the combined treatment model is WBS = 3.31 - 5.2825 x (treatment) + 4.3818 x (total collagen) + 1.1590 x (soluble collagen).

Table 8
Stepwise linear regression equations for combined sides to predict 14 d WBS with treatment included as a variable

	Independent variables	Intercept	β-value	R^2	RMSE	Cp
1	Treatment[a]	30.16	-4.1029	0.36	6.48	-3.18
2	Treatment[a]	33.98	-4.4010	0.39	6.55	-1.51
	Sarcomere length, μm		-2.1772			
3	Treatment[a]	3.31	-5.2825	0.40	6.48	-0.82
	Total collagen, mg/g		4.3818			
	Soluble collagen, %		1.1590			
4	Treatment[a]	-22.51	-3.9927	0.41	6.56	0.90
	Total collagen, mg/g		4.0716			
	Soluble collagen, %		1.1245			
	3 hour pH		4.7472			
5	Treatment[a]	24.03	-3.8285	0.42	6.65	2.62
	Total collagen, mg/g		4.0840			
	Soluble collagen, %		1.1280			
	3 hour pH		5.8498			
	24 hour pH		-9.4205			
6	Treatment[a]	20.50	-3.7028	0.42	6.75	4.41
	Total collagen, mg/g		4.5341			
	Soluble collagen, %		1.1646			
	3 hour pH		6.4372			
	24 hour pH		-9.9751			
	Marbling[b]		0.0036			
7	Treatment[a]	29.30	-4.2826	0.43	6.85	6.16
	Total collagen, mg/g		5.7733			
	Soluble collagen, %		1.2284			
	3 hour pH		5.3749			
	24 hour pH		-9.6808			
	Marbling[b]		0.0060			
	Sarcomere length, μm		-5.2972			
8	Treatment[a]	41.41	-3.8846	0.43	6.97	8.00
	Total collagen, mg/g		5.5340			
	Soluble collagen, %		1.1747			
	3 hour pH		5.7513			
	24 hour pH		-11.9120			
	Marbling[b]		0.0070			
	Sarcomere length, μm		-5.1544			
	Calcium concentration, μM		-0.0018			
9	Treatment[a]	45.86	-3.9337	0.43	7.13	10.00
	Total collagen, mg/g		5.5313			
	Soluble collagen, %		1.1759			
	3 hour pH		5.6242			
	24 hour pH		-12.5268			
	Marbling[b]		0.0067			
	Sarcomere length, μm		-5.1216			
	Calcium concentration, μM		-0.0018			
	MFI[c]		-0.0040			

[a] One side was electrically stimulated (ES); the other side was not (NON-ES).
[b] Moderately Abundant = 800-899, Slightly Abundant= 700-799, Moderate = 600-699, Modest = 500-599,Small = 400-499, Slight = 300-399, Traces = 200-299, and Practically Devoid = 100-199
[c] Myofibrillar fragmentation index

Table 9 shows the stepwise regression equations for the combined treatments with treatment excluded as a variable. 3 h postmortem pH is the single best indicator of tenderness ($R^2 = 0.28$); this is most likely due to the relationship between treatment and early post-mortem pH that is seen in table 2. It is also important to note that the other variable that was affected by treatment is calcium concentration, and it is also appear early in the prediction equations as a valid variable. It could be that 3 h pH and calcium concentration are not used in the best equation from table 8 because they are explained in that particular equation by treatment. Thus when treatment is removed they appear at an earlier stage is the stepwise regression equations.

The best two-variable equation is 3 h pH and 24 h postmortem pH ($R^2 = 0.29$). The best three-variable equation is 3 h and 24 h postmortem pH and calcium concentration ($R^2 = 0.32$). The best equation in this model is the four-variable equation of 3 h and 24 h postmortem pH, calcium concentration and marbling ($R^2 = 0.34$). Thus the best prediction equation for the combined model with treatment excluded as a variable is WBS = 22.52 + 7.9658 x (3 hour pH) – 6.3710 x (24 hour pH) – 0.0053 x (calcium concentration) + 0.0024 x (marbling).

Table 9
Stepwise linear regression equations for combined sides to predict 14 d WBS with treatment excluded as a variable

	Independent variables	Intercept	β-value	R^2	RMSE	Cp
1	3 hour pH	-21.43	9.0340	0.28	6.89	-1.42
2	3 hour pH	-6.33	9.3475	0.29	6.92	-0.07
	24 hour pH		-3.0077			
3	3 hour pH	22.47	7.8701	0.32	6.90	0.86
	24 hour pH		-6.0945			
	Calcium concentration, µM		-0.0051			
4	3 hour pH	22.52	7.9658	0.34	6.94	2.25
	24 hour pH		-6.3710			
	Calcium concentration, µM		-0.0053			
	Marbling[a]		0.0024			
5	3 hour pH	24.32	7.7552	0.35	7.00	3.79
	24 hour pH		-6.1865			
	Calcium concentration, µM		-0.0056			
	Marbling[a]		0.0031			
	Sarcomere length, µm		-0.9770			
6	3 hour pH	45.36	13.0737	0.36	7.08	5.36
	24 hour pH		-19.0284			
	Calcium concentration, µM		-0.0050			
	Marbling[a]		0.0088			
	Total collagen, mg/g		2.5230			
	Soluble collagen, %		0.7034			
7	3 hour pH	55.05	13.6441	0.37	7.17	7.03
	24 hour pH		-20.2284			
	Calcium concentration, µM		-0.0047			
	Marbling[a]		0.0103			
	Total collagen, mg/g		3.4380			
	Soluble collagen, %		0.8322			
	Sarcomere length, µm		-6.2766			
8	3 hour pH	46.39	13.6953	0.37	7.32	9.00
	24 hour pH		-18.8807			
	Calcium concentration, µM		-0.0046			
	Marbling[a]		0.0109			
	Total collagen, mg/g		3.4925			
	Soluble collagen, %		0.8380			
	Sarcomere length, µm		-6.3115			
	MFI[b]		0.0075			

[a] Moderately Abundant = 800-899, Slightly Abundant= 700-799, Moderate = 600-699, Modest = 500-599,Small = 400-499, Slight = 300-399, Traces = 200-299, and Practically Devoid = 100-199
[b] Myofibrillar fragmentation index

CHAPTER V

SUMMARY

Electrical stimulation of steaks resulted in a higher concentration of free calcium in the sarcoplasm as well as a lower pH at 3 hours postmortem. There may also be some influence of electrical stimulation on the solubility of collagen. Calcium concentration correlated to Warner-Bratzler shear force values, as well as pH at 3 hours postmortem, and most likely with pH at 24 hours post mortem. Total collagen correlated with marbling. Myofibrillar fragmentation index correlated with pH at 24 hours postmortem, and pH at 3 hours postmortem correlated with the Warner-Bratzler shear force value.

Prediction equations were formed in order to optimize the explanation of the variation of tenderness. The best prediction equation included treatment, total collagen and soluble collagen ($R^2 = 0.40$), with treatment having the greatest predictability power ($R^2 = 0.36$). This equation represented a significant explanation of the variation in tenderness. Collagen solubility and calcium concentration did not seem to improve the amount of tenderness that was explained. More research is needed to further understand the factors that affect tenderness.

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APPENDIX A

Table A1
Analysis of variance of calcium concentration

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	1037110.8	345704	3.9935
Error	34	2943252.1	86566	Prob > F
C. Total	37	3980362.9		0.0154*

Table A2
Effect tests of calcium concentration

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	907900.98	10.4879	0.0027*
Tenderness value	1	1	14571.99	0.1683	0.6842
Treatment x Tenderness value	1	1	23457.06	0.2710	0.6061

Table A3
Analysis of variance of total collagen

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.440487	0.146829	0.3662
Error	30	12.027861	0.400929	Prob > F
C. Total	33	12.468349		0.7779

Table A4
Effect tests of total collagen

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	0.01351523	0.0337	0.8556
Tenderness value	1	1	0.00725245	0.0181	0.8939
Treatment x Tenderness value	1	1	0.23641481	0.5897	0.4485

Table A5
Analysis of variance of soluble collagen

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	17.03691	5.67897	0.6870
Error	30	247.99730	8.26658	Prob > F
C. Total	33	265.03421		0.5671

Table A6
Effect tests of soluble collagen

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	16.546047	2.0016	0.1674
Tenderness value	1	1	0.278478	0.0337	0.8556
Treatment x Tenderness value	1	1	6.684636	0.8086	0.3757

Table A7
Analysis of variance of marbling

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	2343.46	781.2	0.0425
Error	34	624332.86	18362.7	Prob > F
C. Total	37	626676.32		0.9881

Table A8
Effect tests of marbling

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	3.0451	0.0002	0.9898
Tenderness value	1	1	1579.8872	0.0860	0.7711
Treatment x Tenderness value	1	1	550.4135	0.0300	0.8636

Table A9
Analysis of variance of sarcomere length

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.04273126	0.014244	0.8776
Error	32	0.51934374	0.016229	Prob > F
C. Total	35	0.56207500		0.4630

Table A10
Effect tests of sarcomere length

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	0.01533891	0.9451	0.3383
Tenderness value	1	1	0.02301127	1.4179	0.2425
Treatment x Tenderness value	1	1	0.00605626	0.3732	0.5456

Table A11
Analysis of variance of MFI

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	347.709	115.90	0.0796
Error	34	49484.110	1455.42	Prob > F
C. Total	37	49831.819		0.9706

Table A12
Effect tests of MFI

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	4.82164	0.0033	0.9544
Tenderness value	1	1	292.28687	0.2008	0.6569
Treatment x Tenderness value	1	1	53.36111	0.0367	0.8493

Table A13
Analysis of variance of WBS

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	1410.4597	470.153	13.9901
Error	34	1142.6126	33.606	Prob > F
C. Total	37	2553.0722		<.0001*

Table A14
Effect tests of WBS

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	134.21709	3.9938	0.0537
Tenderness value	1	1	261.56267	7.7832	0.0086*
Treatment x Tenderness value	1	1	509.22237	15.1526	0.0004*

Table A15
Analysis of variance of 3 hour pH

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	3.2167259	1.07224	17.5814
Error	34	2.0735714	0.06099	Prob > F
C. Total	37	5.2902974		<.0001*

Table A16
Effect tests of 3 hour pH

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	2.3784254	38.9986	<.0001*
Tenderness value	1	1	0.0100874	0.1654	0.6868
Treatment x Tenderness value	1	1	0.0050359	0.0826	0.7756

Table A17
Analysis of variance of 24 hour pH

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	0.02025248	0.006751	0.6181
Error	34	0.37136857	0.010923	Prob > F
C. Total	37	0.39162105		0.6081

Table A18
Effect tests of 24 hour pH

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	1	1	0.00514662	0.4712	0.4971
Tenderness value	1	1	0.00069534	0.0637	0.8023
Treatment x Tenderness value	1	1	0.00514662	0.4712	0.4971

APPENDIX B

Table 27
Pairwise correlations of ES sides

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
Tot Col	Ca Conc µM	-0.2216	18	-0.6239	0.2736	0.3769
% Sol Col	Ca Conc µM	0.1198	18	-0.3676	0.5556	0.6357
% Sol Col	Tot Col	-0.8111	18	-0.9270	-0.5541	<.0001*
MARBL	Ca Conc µM	0.3140	19	-0.1636	0.6723	0.1905
MARBL	Tot Col	-0.4910	18	-0.7793	-0.0313	0.0385*
MARBL	% Sol Col	0.1923	18	-0.3017	0.6048	0.4447
SARC	Ca Conc µM	0.1265	18	-0.3618	0.5603	0.6170
SARC	Tot Col	-0.0439	17	-0.5137	0.4462	0.8672
SARC	% Sol Col	-0.0651	17	-0.5292	0.4289	0.8039
SARC	MARBL	0.2449	18	-0.2507	0.6387	0.3274
MFI	Ca Conc µM	0.0014	19	-0.4531	0.4553	0.9956
MFI	Tot Col	0.1649	18	-0.3272	0.5866	0.5132
MFI	% Sol Col	-0.0983	18	-0.5403	0.3863	0.6981
MFI	MARBL	-0.1926	19	-0.5948	0.2867	0.4296
MFI	SARC	-0.0448	18	-0.5012	0.4310	0.8597
WBS N	Ca Conc µM	-0.2037	19	-0.6022	0.2761	0.4030
WBS N	Tot Col	0.0487	18	-0.4279	0.5041	0.8479
WBS N	% Sol Col	0.2825	18	-0.2123	0.6621	0.2560
WBS N	MARBL	-0.3841	19	-0.7138	0.0849	0.1045
WBS N	SARC	-0.2497	18	-0.6417	0.2459	0.3178
WBS N	MFI	0.2065	19	-0.2734	0.6040	0.3964
pH3	Ca Conc µM	-0.0922	19	-0.5245	0.3778	0.7073
pH3	Tot Col	0.1100	18	-0.3762	0.5487	0.6639
pH3	% Sol Col	0.0137	18	-0.4561	0.4775	0.9570
pH3	MARBL	-0.3518	19	-0.6950	0.1219	0.1397
pH3	SARC	-0.0001	18	-0.4669	0.4668	0.9998
pH3	MFI	-0.3066	19	-0.6678	0.1715	0.2018
pH3	WBS N	0.0664	19	-0.3999	0.5054	0.7871
pH24	Ca Conc µM	-0.2442	19	-0.6287	0.2362	0.3138
pH24	Tot Col	0.1451	18	-0.3452	0.5731	0.5657
pH24	% Sol Col	-0.1003	18	-0.5418	0.3846	0.6922
pH24	MARBL	0.0174	19	-0.4403	0.4679	0.9436
pH24	SARC	-0.2038	18	-0.6124	0.2907	0.4173
pH24	MFI	-0.4744	19	-0.7640	-0.0257	0.0402*
pH24	WBS N	-0.0618	19	-0.5019	0.4038	0.8017
pH24	pH3	0.6743	19	0.3173	0.8639	0.0015*

Table 28
Pairwise comparisons of NON-ES sides

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
Tot Col 2	Ca Conc µM 2	0.1372	16	-0.3847	0.5926	0.6125
% Sol Col 2	Ca Conc µM 2	-0.2534	16	-0.6655	0.2771	0.3437
% Sol Col 2	Tot Col 2	-0.9117	16	-0.9693	-0.7589	<.0001*
MARBL 2	Ca Conc µM 2	0.2057	19	-0.2741	0.6035	0.3981
MARBL 2	Tot Col 2	-0.3193	16	-0.7036	0.2096	0.2281
MARBL 2	% Sol Col 2	0.4211	16	-0.0943	0.7585	0.1043
SARC 2	Ca Conc µM 2	0.2748	18	-0.2204	0.6573	0.2698
SARC 2	Tot Col 2	0.2008	15	-0.3472	0.6465	0.4731
SARC 2	% Sol Col 2	-0.2137	15	-0.6543	0.3353	0.4444
SARC 2	MARBL 2	0.1593	18	-0.3323	0.5828	0.5279
MFI 2	Ca Conc µM 2	-0.0229	19	-0.4722	0.4359	0.9260
MFI 2	Tot Col 2	-0.1872	16	-0.6249	0.3401	0.4875
MFI 2	% Sol Col 2	0.1118	16	-0.4064	0.5756	0.6802
MFI 2	MARBL 2	-0.3131	19	-0.6718	0.1645	0.1918
MFI 2	SARC 2	-0.0078	18	-0.4730	0.4607	0.9755
WBS N 2	Ca Conc µM 2	-0.0232	19	-0.4724	0.4356	0.9249
WBS N 2	Tot Col 2	-0.0495	16	-0.5322	0.4574	0.8554
WBS N 2	% Sol Col 2	0.0753	16	-0.4367	0.5505	0.7815
WBS N 2	MARBL 2	0.0634	19	-0.4024	0.5032	0.7964
WBS N 2	SARC 2	0.1447	18	-0.3455	0.5729	0.5668
WBS N 2	MFI 2	-0.0590	19	-0.4998	0.4061	0.8104
pH3 2	Ca Conc µM 2	0.0899	19	-0.3798	0.5228	0.7144
pH3 2	Tot Col 2	0.0887	16	-0.4257	0.5598	0.7438
pH3 2	% Sol Col 2	-0.1632	16	-0.6096	0.3618	0.5460
pH3 2	MARBL 2	-0.2164	19	-0.6106	0.2638	0.3736
pH3 2	SARC 2	-0.3579	18	-0.7067	0.1309	0.1448
pH3 2	MFI 2	-0.1396	19	-0.5584	0.3359	0.5685
pH3 2	WBS N 2	0.0289	19	-0.4310	0.4768	0.9067
pH24 2	Ca Conc µM 2	-0.3729	19	-0.7073	0.0979	0.1159
pH24 2	Tot Col 2	-0.1107	16	-0.5748	0.4074	0.6833
pH24 2	% Sol Col 2	0.1040	16	-0.4130	0.5703	0.7016
pH24 2	MARBL 2	-0.0479	19	-0.4914	0.4154	0.8456
pH24 2	SARC 2	-0.0160	18	-0.4793	0.4542	0.9497
pH24 2	MFI 2	-0.4348	19	-0.7424	0.0242	0.0629
pH24 2	WBS N 2	0.0791	19	-0.3891	0.5148	0.7476
pH24 2	pH3 2	0.1523	19	-0.3243	0.5673	0.5336

Table 29
Pairwise correlations combined data

Variable	by Variable	Correlation	Count	Lower 95%	Upper 95%	Signif Prob
Tot Col	Ca Conc µM	-0.0539	34	-0.3850	0.2896	0.7623
% Sol Col	Ca Conc µM	0.1185	34	-0.2289	0.4390	0.5046
% Sol Col	Tot Col	-0.7995	34	-0.8955	-0.6322	<0.0001*
MARBL	Ca Conc µM	0.2151	38	-0.1123	0.5004	0.1946
MARBL	Tot Col	-0.4166	34	-0.6616	-0.0913	0.0143*
MARBL	% Sol Col	0.2644	34	-0.0810	0.5531	0.1308
SARC M	Ca Conc µM	0.2056	36	-0.1319	0.5003	0.2291
SARC M	Tot Col	0.0213	32	-0.3298	0.3673	0.9077
SARC M	% Sol Col	-0.0879	32	-0.4236	0.2690	0.6324
SARC M	MARBL	0.2026	36	-0.1349	0.4980	0.2359
MFI	Ca Conc µM	-0.0080	38	-0.3268	0.3125	0.9622
MFI	Tot Col	0.0097	34	-0.3295	0.3468	0.9565
MFI	% Sol Col	-0.0151	34	-0.3515	0.3247	0.9325
MFI	MARBL	-0.2506	38	-0.5280	0.0751	0.1292
MFI	SARC M	-0.0345	36	-0.3590	0.2974	0.8417
WBS Newtons	Ca Conc µM	-0.3232	38	-0.5827	-0.0039	0.0478*
WBS Newtons	Tot Col	-0.0778	34	-0.4053	0.2674	0.6618
WBS Newtons	% Sol Col	0.0117	34	-0.3278	0.3485	0.9477
WBS Newtons	MARBL	-0.0654	38	-0.3772	0.2597	0.6966
WBS Newtons	SARC M	-0.1080	36	-0.4216	0.2286	0.5307
WBS Newtons	MFI	0.0358	38	-0.2871	0.3515	0.8308
pH3	Ca Conc µM	-0.4063	38	-0.6425	-0.0995	0.0114*
pH3	Tot Col	-0.0306	34	-0.3650	0.3108	0.8637
pH3	% Sol Col	-0.1856	34	-0.4929	0.1627	0.2932
pH3	MARBL	-0.1620	38	-0.4579	0.1663	0.3313
pH3	SARC M	-0.1956	36	-0.4925	0.1421	0.2530
pH3	MFI	-0.1357	38	-0.4364	0.1924	0.4167
pH3	WBS Newtons	0.4112	38	0.1054	0.6460	0.0103*
pH24	Ca Conc µM	-0.3175	38	-0.5785	0.0025	0.0521
pH24	Tot Col	0.0108	34	-0.3285	0.3477	0.9515
pH24	% Sol Col	-0.0438	34	-0.3764	0.2988	0.8058
pH24	MARBL	-0.0151	38	-0.3332	0.3060	0.9281
pH24	SARC M	-0.1414	36	-0.4491	0.1962	0.4106
pH24	MFI	-0.4393	38	-0.6655	-0.1392	0.0058*
pH24	WBS Newtons	0.1258	38	-0.2020	0.4283	0.4517
pH24	pH3	0.3832	38	0.0724	0.6262	0.0176*

Appendix C

Table 30
Complete data set

Calf ID	FAM	Ca Conc µM	Treat	Tot Col	% Sol Col	MARBL	SARC	MFI	WBS N	pH3	pH24
115P	81	1131.617	ES	2.2067	16.176	330	1.6	84.5	22.35328	5.19	5.45
129P	81	661.4643	ES			400	1.74	111	36.41699	5.07	5.42
132P	72	917.5905	ES	2.2619	13.415	670	1.85	138.5	23.84781	5.27	5.52
137P	81	448.3526	ES	3.1437	13.747	300	1.67	119	27.03693	5.19	5.52
2/40	72	489.0059	ES	2.0703	17.207	430	1.7	37	25.84346	5.9	5.81
2/47	75	580.3695	ES	4.4967	5.7239	370	1.76	65.25	23.05053	5.64	5.73
2/50	75	702.2868	ES	3.4936	10.709	310	1.29	33	30.39473	5.45	5.68
2/55	74	349.5412	ES	3.1891	9.4002	320	1.8	25.75	28.62659	5.61	5.59
2/58	72	876.8132	ES	2.4216	18.131	340		33	31.42639	5.9	5.58
2/62	75	578.2053	ES	2.9457	12.263	330	1.98	53.25	22.72495	5.48	5.56
2/67	74	432.2268	ES	1.9827	17.646	340	1.67	42	34.60178	5.59	5.66
2/75	75	1759.225	ES	2.8901	11.694	430	1.97	42.75	25.39236	5.54	5.58
802N	77	1453.268	ES	1.9961	14.817	620	1.76	19.25	24.63627	5.3	5.57
803N	70	504.399	ES	2.6491	15.888	540	1.89	17.5	32.07853	5.18	5.63
807N	77	950.8105	ES	2.597	15.998	640	1.75	14	23.40259	5.22	5.57
808N	70	453.2249	ES	2.2306	11.916	590	1.75	16.75	21.42165	5.27	5.59
839N	76	741.0201	ES	2.2658	13.846	520	1.79	14.25	19.23672	5.37	5.59
846N	76	869.3531	ES	1.8688	15.108	620	1.69	13.75	21.28239	5.43	5.68
847N	76	478.1632	ES	2.9662	12.542	390	1.91	15.5	21.33241	5.38	5.55
115P	81	420.5619	NON	1.8908	15.247	340	1.63	145	30.12897	5.94	5.45
129P	81	356.0129	NON			410	1.62	84	21.55207	6.11	5.4
132P	72	295.956	NON	2.1233	15.715	640	1.86	96	41.7067	5.5	5.6
137P	81	851.3541	NON	2.9827	9.5058	290	1.82	86.5	39.44529	6.18	5.56
2/40	72	385.7665	NON	2.0556	13.591	450	1.89	44	25.8366	6.2	5.8
2/47	75	282.61	NON			380	1.49	53	32.24034	6.46	5.84
2/50	75	213.0234	NON			310	1.72	54.5	51.27309	6	5.71
2/55	74	240.827	NON	2.9628	10.128	370	1.74	40	35.95608	5.89	5.65
2/58	72	671.2664	NON	2.7909	11.133	360	1.75	34.75	31.89319	6.1	5.58
2/62	75	461.1351	NON	3.5017	8.8636	330	1.75	44.5	42.38434	6	5.58
2/67	74	243.238	NON	1.8846	13.621	320	1.68	49.5	36.64255	5.75	5.73
2/75	75	439.6957	NON	2.6784	10.8	440	1.75	55	21.35986	5.87	5.62
802N	77	569.9647	NON	1.8805	14.574	650	1.71	12.25	54.37101	6.13	5.55
803N	70	419.1357	NON	3.6477	8.913	480		12.75	27.4851	5.71	5.64
807N	77	361.7841	NON	2.7656	12.568	570	1.7	11.5	37.28194	6.48	5.62
808N	70	526.9856	NON	2.1296	15.359	630	1.71	15	34.92638	5.71	5.71
839N	76	517.2921	NON	2.0924	14.565	500	1.7	9.25	22.50822	5.88	5.57
846N	76	660.3206	NON	2.2688	12.397	740	1.72	45	31.80591	5.95	5.63
847N	76	298.4239	NON	2.294	14.485	370	1.68	12.35	32.21779	6.15	5.78

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